

CLAIMS

1. An alkanethiol of formula (I):



wherein -L- is $-(\text{A}_x-\text{B}_y-\text{E}_z-\text{D})_w$;

each A, B, E and D are individually $\text{C}(\text{R}_\text{A}\text{R}_\text{A}')$ -, $-\text{C}(\text{R}_\text{B}\text{R}_\text{B}')$ -, $-\text{C}(\text{R}_\text{E}\text{R}_\text{E}')$ -, and $-\text{C}(\text{R}_\text{D}\text{R}_\text{D}')$ -, respectively;

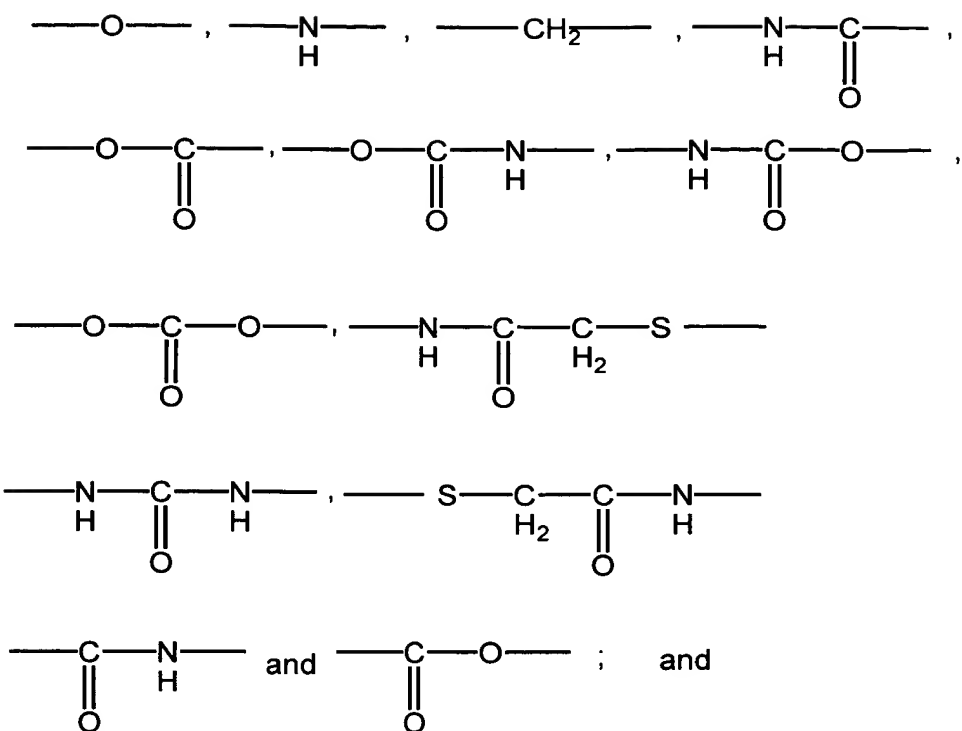
each R_A , R_B , R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A , R_B , R_E and R_D together form a bond, or any two of R_A , R_B , R_E and R_D together with the atoms to which they are bonded form a ring;

each R_A' , R_B' , R_E' and R_D' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A' , R_B' , R_E' and R_D' together form a bond, or any two R_A' , R_B' , R_E' and R_D' together with the atoms to which they are bonded form a ring;

each x, y and z are individually either 0 or 1;

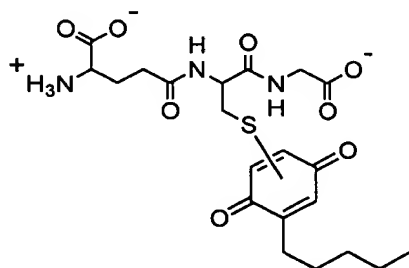
w is 1 to 5;

-Q- is selected from the group consisting of



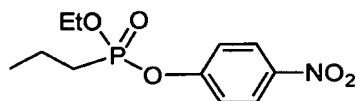
-T comprises a reactant ligand.

2. The alkanethiol of claim 1, wherein -L- contains 8 to 18 carbon atoms.
3. The alkanethiol of claim 1, wherein -L- is an alkylene containing 6 to 18 carbon atoms, and -Q- is -O-.
4. The alkanethiol of claim 1, wherein -Q- is -O- or -CH₂-.
5. The alkanethiol of claim 1, wherein the reactant ligand is a moiety of formula (II)



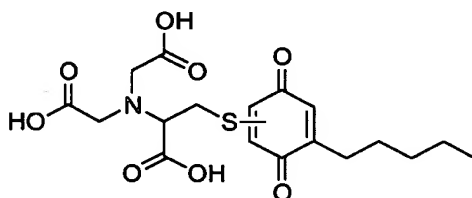
(II).

6. The alkanethiol of claim 1, wherein the reactant ligand is a moiety of formula (III)



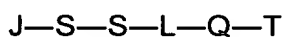
(III).

7. The alkanethiol of claim 1, wherein the reactant ligand is a moiety of formula (IV)



(IV).

8. A disulfide of formula (V):



(V),

wherein -L- is $-(A_x-B_y-E_z-D)_w$;

each A, B, E and D are individually $C(R_A R_A')$ -, $-C(R_B R_B')$ -, $-C(R_E R_E')$ -, and $-C(R_D R_D')$ -, respectively;

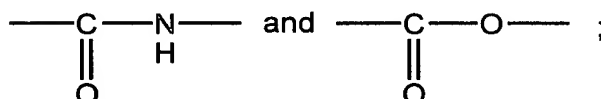
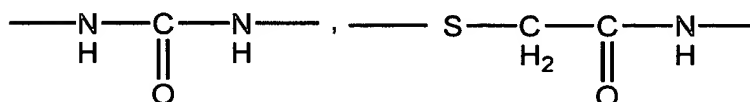
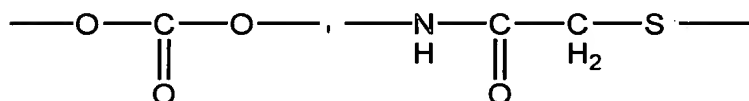
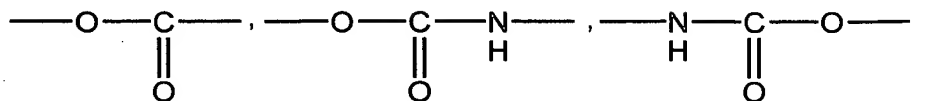
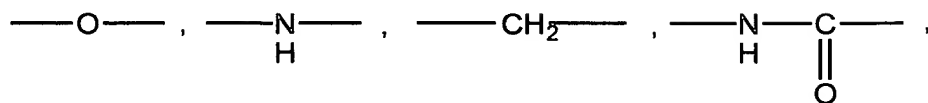
each R_A , R_B , R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A , R_B , R_E and R_D together form a bond, or any two of R_A , R_B , R_E and R_D together with the atoms to which they are bonded form a ring;

each R_A' , R_B' , R_E' and R_D' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A' , R_B' , R_E' and R_D' together form a bond, or any two R_A' , R_B' , R_E' and R_D' together with the atoms to which they are bonded form a ring;

each x, y and z are individually either 0 or 1;

w is 1 to 5;

-Q- is selected from the group consisting of



-T is a reactant ligand;

-J is selected from the group consisting of H, halogen, R, -OR,

-NRR', -C(O)R, and -C(O)OR;

R is selected from the group consisting of alkyl, alkenyl, alkynyl, aryl and heterocyclic radical; and

R' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical;

wherein the disulfide does not selectively bind avidin or streptavidin.

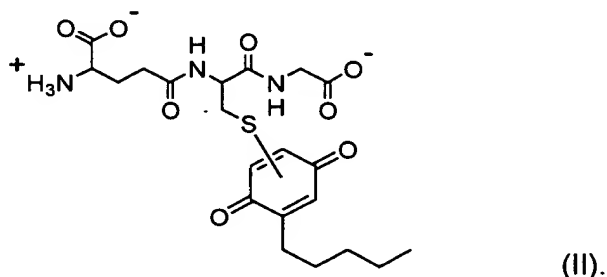
9. The disulfide of claim 8, wherein-J is a moiety of formula (VI)
 $\text{---}(\text{CH}_2)_c(\text{OCH}_2\text{CH}_2)_n\text{OH}$ (VI),
 wherein c is 2 to 20, and n is 2 to 10.

10. The disulfide of claim 8, wherein -L- contains 8 to 18 carbon atoms.

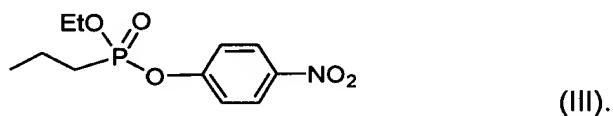
11. The disulfide of claim 8, wherein -Q- is -O- or -CH₂-.

12. The disulfide of claim 8, wherein -L- is an alkylene containing 6 to 18 carbon atoms, and -Q- is -O-.

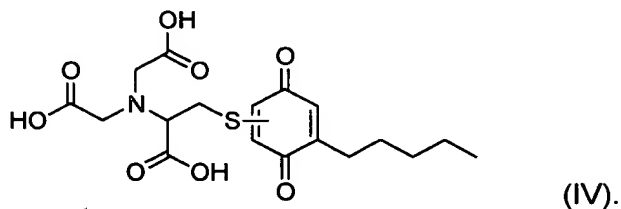
13. The disulfide of claim 8, wherein the reactant ligand is a moiety of formula (II)



14. The disulfide of claim 8, wherein the reactant ligand is a moiety of formula (III)



15. The disulfide of claim 8, wherein the reactant ligand is a moiety of formula (IV)



16. A substrate, comprising:
(i) a surface comprising gold, and
(ii) a plurality of moieties, on at least a portion of said surface,

wherein said moieties are alkanethiolate moieties of formula

(VII):



wherein -L- is $-(\text{A}_x-\text{B}_y-\text{E}_z-\text{D})_w$;

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each A, B, E and D are individually $\text{C}(\text{R}_\text{A}\text{R}_\text{A}')$ -, $-\text{C}(\text{R}_\text{B}\text{R}_\text{B}')$ -, $-\text{C}(\text{R}_\text{E}\text{R}_\text{E}')$ -, and $-\text{C}(\text{R}_\text{D}\text{R}_\text{D}')$ -, respectively;

each R_A , R_B , R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A , R_B , R_E and R_D together form a bond, or any two of R_A , R_B , R_E and R_D together with the atoms to which they are bonded form a ring;

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each R_A' , R_B' , R_E' and R_D' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A' , R_B' , R_E' and R_D' together form a bond, or any two R_A' , R_B' , R_E' and R_D' together with the atoms to which they are bonded form a ring;

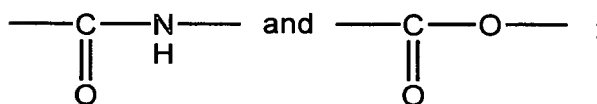
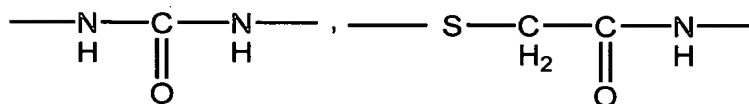
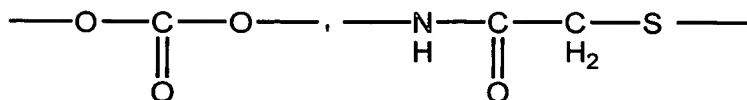
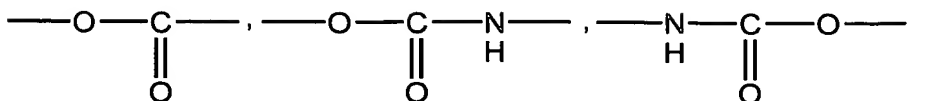
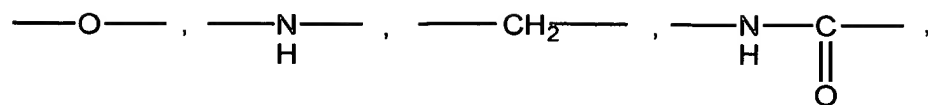
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each x, y and z are individually either 0 or 1;

w is 1 to 5;

-Q- is selected from the group consisting of

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-T comprises a reactant ligand; and

Surf designates where the moieties attach to said surface.

17. The substrate of claim 16, further comprising:
(iii) a patterned monolayer comprising said moieties.

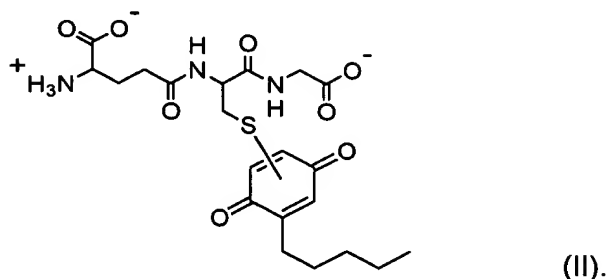
18. The substrate of claim 16, further comprising:
(iv) a surface layer,
wherein said surface layer is on said substrate.

19. The substrate of claim 16, wherein -L- contains 8 to 18 carbon atoms.

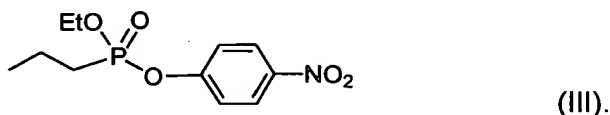
20. The substrate of claim 16, wherein -Q- is -O- or -CH₂-.

21. The substrate of claim 16, wherein -L- is an alkylene containing 6 to 18 carbon atoms, and -Q- is -O-.

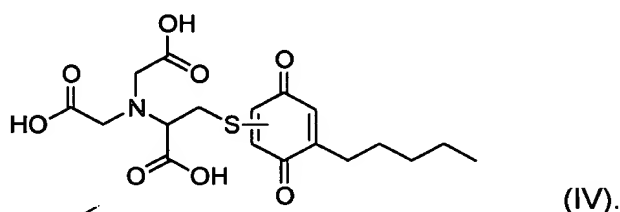
22. The substrate of claim 16, wherein the reactant ligand is a moiety of formula (II)



23. The substrate of claim 16, wherein the reactant ligand is a moiety of formula (III)



24. The substrate of claim 16, wherein the reactant ligand is a moiety of formula (IV)



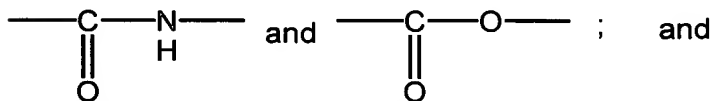
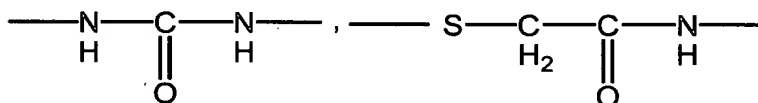
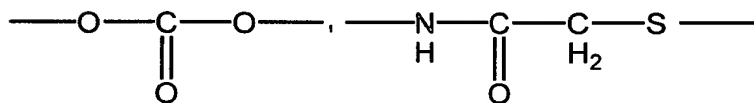
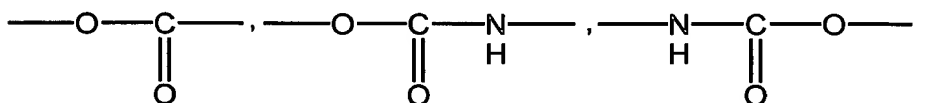
25. A substrate, comprising:
a plurality of reactant ligands, attached to said substrate.

26. The substrate of claim 25, wherein the substrate comprises at least one member selected from the group consisting of metal, metal oxide, glass, ceramic, quartz, silicon, polymer, sepharose, agarose, a colloid, a lipid bilayer, and a lipid monolayer.

27. The substrate of claim 26, wherein the substrate comprises gold.

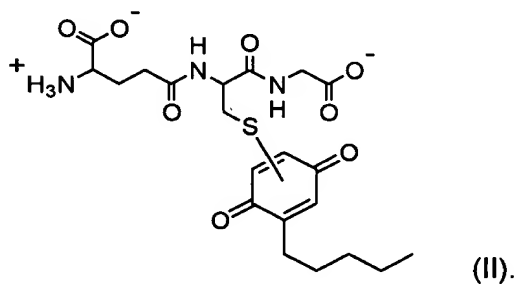
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- $$-Q-T \quad (VIII);$$

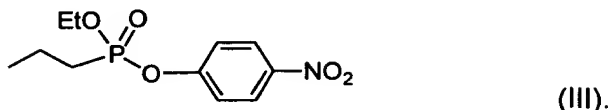
$$\text{—O—}, \text{—}\underset{\text{H}}{\text{N}}\text{—}, \text{—CH}_2\text{—}, \text{—}\underset{\text{H}}{\text{N}}\text{—}\underset{\text{O}}{\underset{\parallel}{\text{C}}}\text{—},$$


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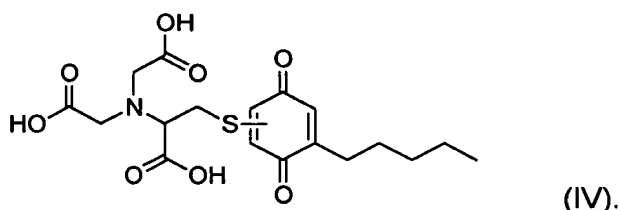
29. The substrate of claim 28, wherein the reactant ligand is a moiety of formula (II)



30. The substrate of claim 28, wherein the reactant ligand is a moiety of formula (III)



31. The substrate of claim 28, wherein the reactant ligand is a moiety of formula (IV)



32. The substrate of claim 25, comprising:

- (i) a surface on the substrate, and
- (ii) a plurality of moieties, on at least a portion of said surface, wherein said moieties are moieties of formula (IX):



wherein -L- is $-(A_x-B_y-E_z-D)_w$;

each A, B, E and D are individually $C(R_A R_{A'})$ -, $-C(R_B R_{B'})$ -, $-C(R_E R_{E'})$ -, and $-C(R_D R_{D'})$ -, respectively;

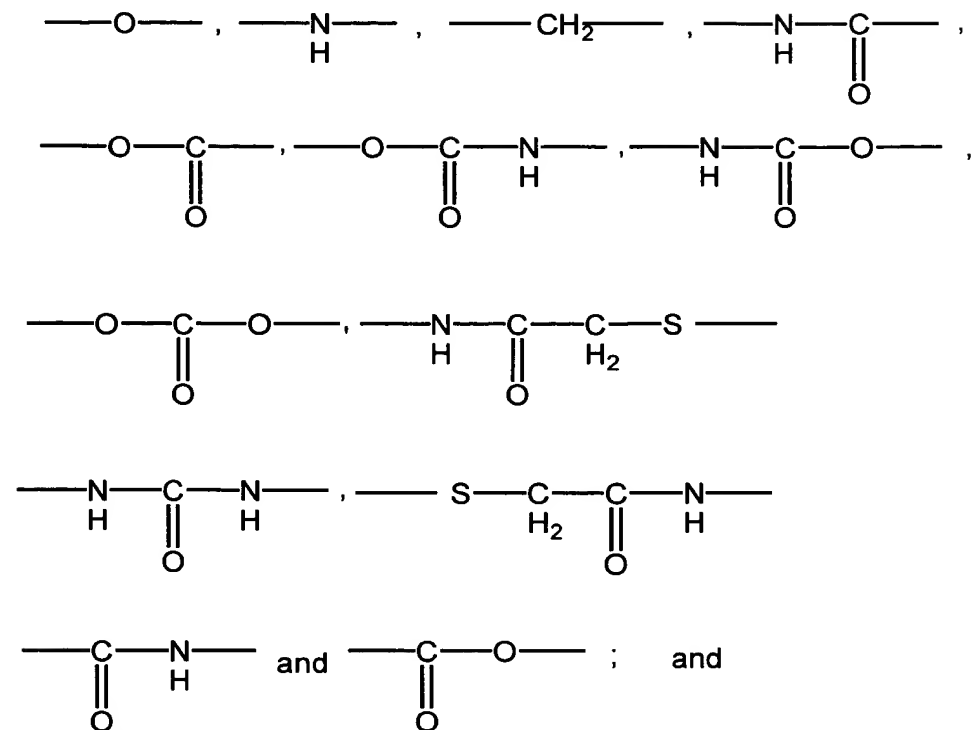
each R_A , R_B , R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A , R_B , R_E and R_D together form a bond, or any two of R_A , R_B , R_E and R_D together with the atoms to which they are bonded form a ring;

each $R_{A'}$, $R_{B'}$, $R_{E'}$ and $R_{D'}$ is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of $R_{A'}$, $R_{B'}$, $R_{E'}$ and $R_{D'}$ together form a bond, or any two $R_{A'}$, $R_{B'}$, $R_{E'}$ and $R_{D'}$ together with the atoms to which they are bonded form a ring;

each x, y and z are individually either 0 or 1;

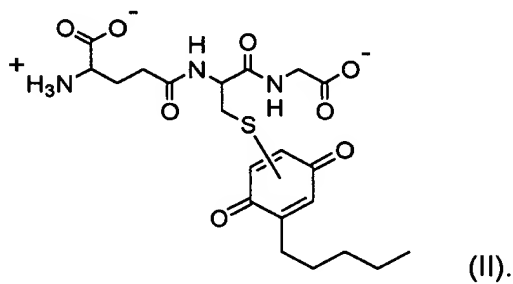
w is 1 to 5;

-Q- is selected from the group consisting of

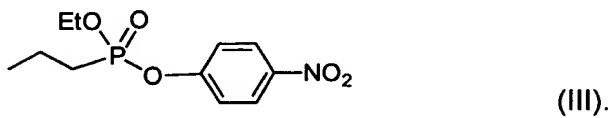


-T comprises the reactant ligand.

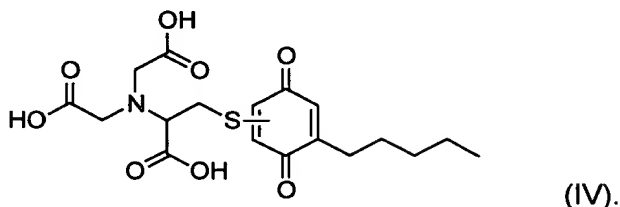
33. The substrate of claim 32, wherein the reactant ligand is a moiety of formula (II)



34. The substrate of claim 32, wherein the reactant ligand is a moiety of formula (III)

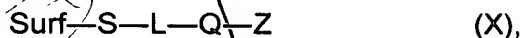


35. The substrate of claim 32, wherein the reactant ligand is a moiety of formula (IV)



36. A protein chip, comprising:
a substrate; and
a reaction product of a reactant ligand and a fusion polypeptide,
on said substrate;
wherein said fusion polypeptide comprises a capture
polypeptide moiety corresponding to said reactant ligand.

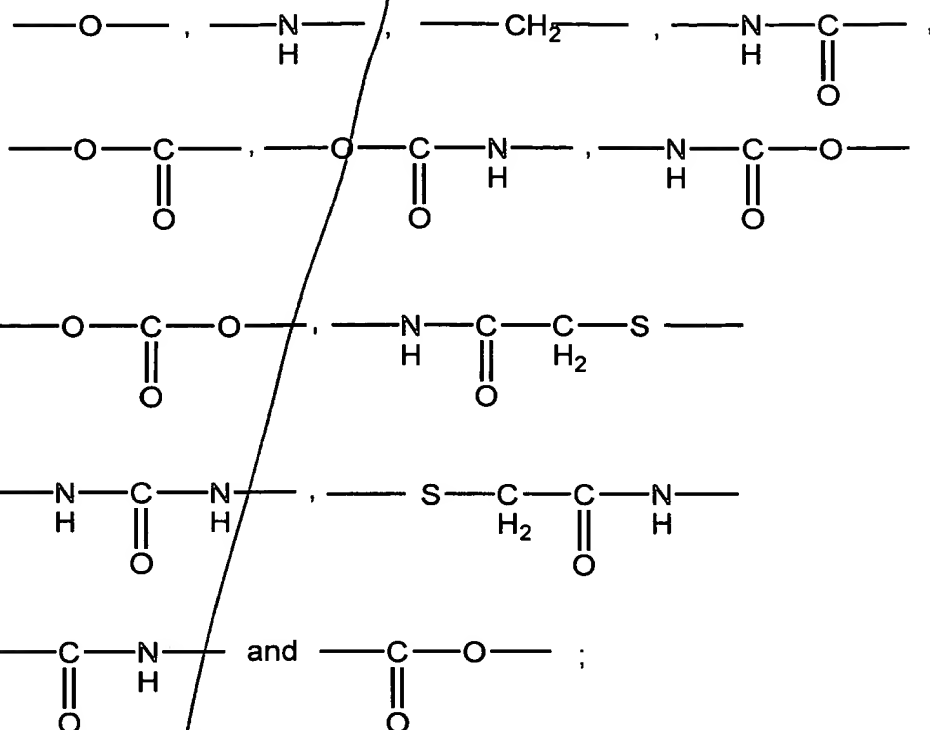
37. The protein chip of claim 36, further comprising:
(i) a surface comprising gold on said substrate, and
(ii) a plurality of moieties, on at least a portion of said surface,
wherein said moieties are alkanethiolate moieties of formula (X):



wherein -L- is $-(A_x-B_y-E_z-D)_w$;
each A, B, E and D are individually $C(R_A R_A')$ -, $-C(R_B R_B')$ -, $-C(R_E R_E')$ -, and $-C(R_D R_D')$ -, respectively;
each R_A , R_B , R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A , R_B , R_E and R_D together form a bond, or any two of R_A , R_B , R_E and R_D together with the atoms to which they are bonded form a ring;
each R_A' , R_B' , R_E' and R_D' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A' , R_B' , R_E' and R_D' together form a bond, or any two R_A' , R_B' , R_E' and R_D' together with the atoms to which they are bonded form a ring;
each x, y and z are individually either 0 or 1;
w is 1 to 5;

whl. of final prod.?

-Q- is selected from the group consisting of



-Z comprises said reaction product; and

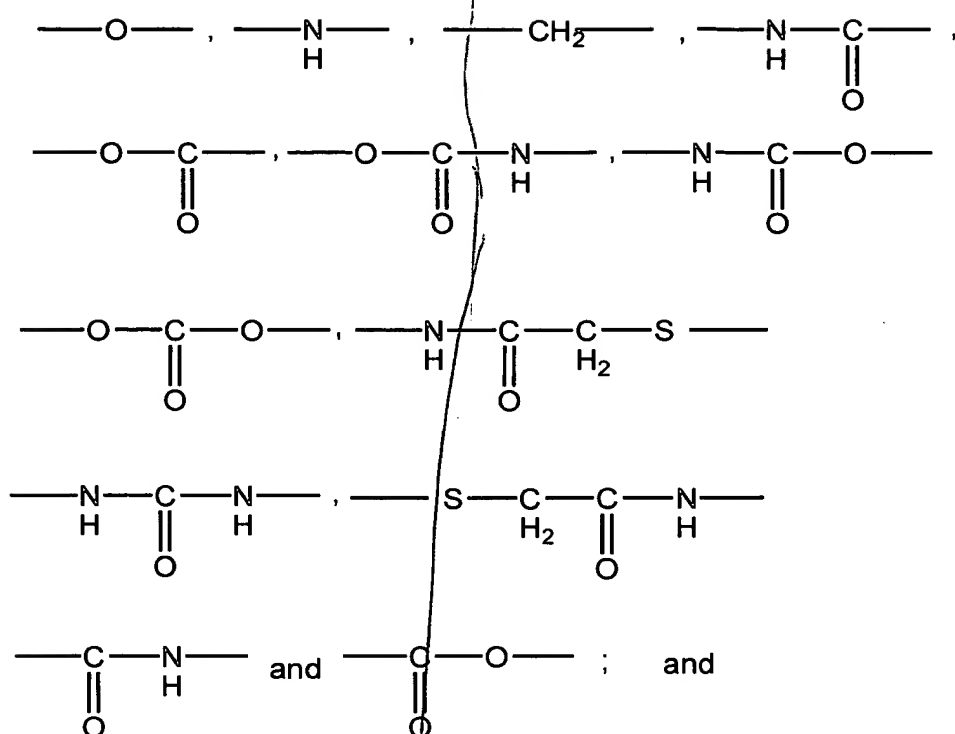
Surf designates where the moieties attach to said surface.

38. The protein chip of claim 36, further comprising:
- (i) a surface on the substrate, and
 - (ii) a plurality of moieties, on at least a portion of said surface, wherein said moieties are moieties of formula (XI):
- $$\text{---Q---Z} \quad (\text{XI}),$$
- wherein -Q- is selected from the group consisting of

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each R_A, R_B, R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A, R_B, R_E and R_D together form a bond, or any two of R_A, R_B, R_E and R_D together with the atoms to which they are bonded form a ring;

each R_A' , R_B' , R_E' and R_D' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A' ,

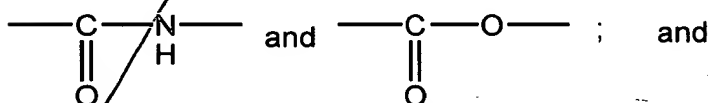
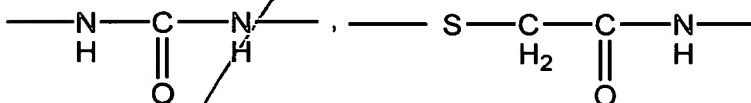
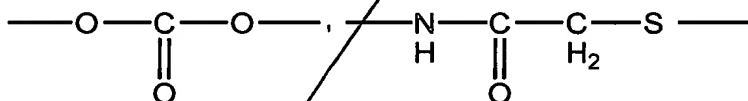
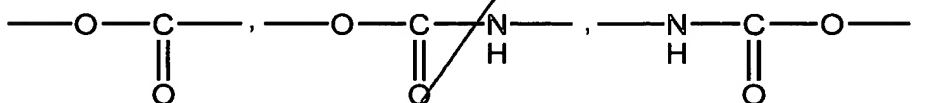
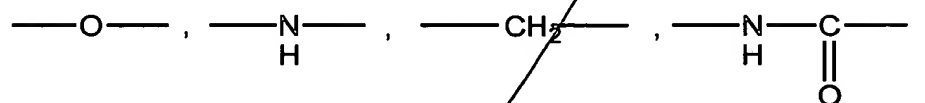
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R_B' , R_E' and R_D' together form a bond, or any two R_A' , R_B' , R_E' and R_D' together with the atoms to which they are bonded form a ring;

each x, y and z are individually either 0 or 1;

w is 1 to 5;

-Q- is selected from the group consisting of



~~Z comprises~~ said reaction product.

40. A method of making a substrate, comprising contacting a surface with the alkanethiol of claim 1;

wherein said surface comprises gold.

41. A method of making a substrate, comprising contacting a surface with the disulfide of claim 8;

wherein said surface comprises gold.

42. A method of making a protein chip, comprising:

contacting a fusion polypeptide with the substrate of claim 16.

43. A method of making a protein chip, comprising:
contacting a fusion polypeptide with the substrate of claim 22;
wherein said fusion polypeptide is a fusion polypeptide of GST.

5 44. A method of making a protein chip, comprising:
contacting a fusion polypeptide with the substrate of claim 23;
wherein said fusion polypeptide is a fusion polypeptide of cutinase.

10 45. A method of making a protein chip, comprising:
contacting a fusion polypeptide with the substrate of claim 24;
wherein said fusion polypeptide is a fusion polypeptide of GGCHHHC.

46. A method of making a protein chip, comprising:
contacting a fusion polypeptide with the substrate of claim 25.

15 47. A method of assaying kinase activity, comprising:
contacting a mixture comprising at least one kinase with the
protein chip of claim 36;
wherein the fusion polypeptide is a fusion polypeptide of a
kinase substrate; and
correlating a change in the kinase substrate with kinase activity.

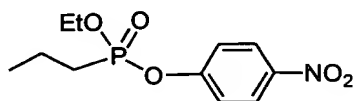
20 48. A method of assaying protease activity, comprising:
contacting a mixture comprising at least one protease with the
protein chip of claim 36;
wherein the fusion polypeptide is a fusion polypeptide of a
protease substrate; and
25 correlating a change in the protease substrate with protease
activity.

49. A fusion of a capture polypeptide and a display moiety,

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wherein the display moiety does not consist of GST, His tag, lacZ, trpE, maltose binding protein, thioredoxin, or F_c region of an immunoglobulin; and

a corresponding reactant ligand of the capture polypeptide is a moiety of formula (III):



(III).

50. An isolated polynucleotide encoding the fusion of claim 49 wherein the fusion is a fusion polypeptide.

51. A vector comprising the polynucleotide of claim 50.

52. A host cell comprising the polynucleotide of claim 50.

53. The cell of claim 52, wherein the cell is prokaryotic.

54. The cell of claim 52, wherein the cell is eukaryotic.

55. The cell of claim 52, wherein the cell is bacterial, insect, plant, fungal or mammalian.

56. The cell of claim 52, wherein the cell is animal.

57. A method of making the vector of claim 46, comprising:
providing a ligation site in the vector,
providing a polynucleotide encoding the fusion polypeptide; and
ligating the vector and the polynucleotide at the ligation site.

58. The method of claim 52, wherein the polynucleotide is operably linked to a promoter of the vector when ligated therein.

59. A method of determining an enzymatic activity of a sample, comprising:

contacting the protein chip of claim 36 with the sample.

60. A method of determining an enzymatic activity of a sample,
comprising:

contacting the protein chip of claim 37 with the sample.

61. A method of determining an enzymatic activity of a sample,
comprising:

contacting the protein chip of claim 38 with the sample.

62. A method of determining an enzymatic activity of a sample,
comprising:

contacting the protein chip of claim 39 with the sample.

63. The method of claim 59, wherein said chip comprises caspase
substrates.

64. The method of claim 59, wherein said chip comprises kinase
substrates.

65. The method of claim 59, wherein said chip comprises protease
substrates.

66. A method of determining the presence of antibodies in a sample,
comprising:

contacting the protein chip of claim 36 with the sample.

67. A method of determining the presence of a plurality of antibodies
in a sample, comprising:

contacting the protein chip of claim 37 with the sample.

68. A method of determining the presence of a plurality of antibodies
in a sample, comprising:

contacting the protein chip of claim 38 with the sample.

69. A method of determining the presence of a plurality of antibodies
in a sample, comprising:

contacting the protein chip of claim 39 with the sample.

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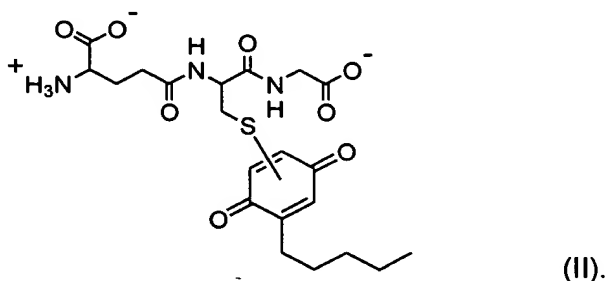
70. The method of claim 66, wherein the chip comprises epitopes associated with a pathological condition or disease.

71. ✓ A method of immobilizing a fusion on a surface, comprising:
reacting a fusion with a reactant ligand;
wherein the reactant ligand is attached to the surface.

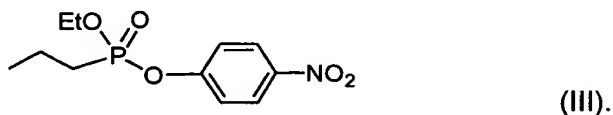
72. The method of claim 71, wherein the fusion comprises a display moiety, and wherein the display moiety is a polypeptide.

73. The method of claim 71, wherein the fusion comprises a display moiety, and wherein the display moiety is a polynucleotide.

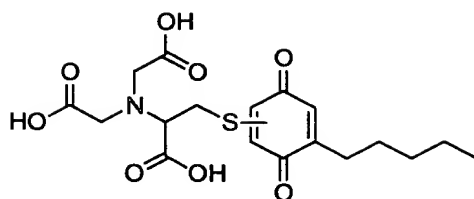
74. The method of claim 71, wherein the reactant ligand is a moiety of formula (II):



75. The method of claim 71, wherein the reactant ligand is a moiety of formula (III):



76. The method of claim 71, wherein the reactant ligand is a moiety of formula (IV):



(IV).

77. The method of claim 71, wherein the surface is selected from the group consisting of sepharose, agarose, polyacrylamide, polystyrene, dextran, lipid monolayer, lipid bilayer, metal, metal oxide, glass, ceramic, quartz, silicon, polyethylene, and polypropylene.

78. The method of claim 71, wherein the surface comprises gold.

79. The method of claim 71, wherein the surface comprises a gel.

80. The method of claim 71, wherein the surface comprises a porous material.

81. A method of immobilizing a display moiety on a surface, comprising:

reacting a capture polypeptide moiety with a corresponding reactant ligand to form a covalent bond;

wherein the capture polypeptide moiety is a fusion with the display moiety; and

wherein the reactant ligand is attached to the surface.

82. The method of claim 81, wherein the half-life of the covalent bond is at least 3 minutes.

83. The method of claim 81, wherein the half-life of the covalent bond is at least 30 minutes.

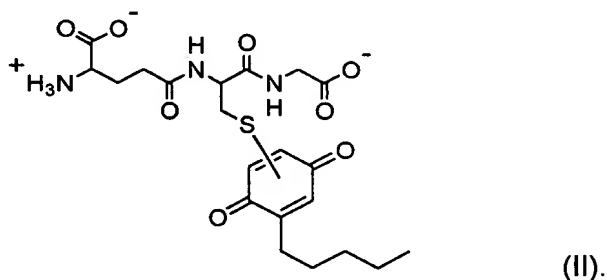
84. The method of claim 81, wherein the half-life of the covalent bond is at least 1 hour.

85. The method of claim 81, wherein the half-life of the covalent bond is at least 24 hours.

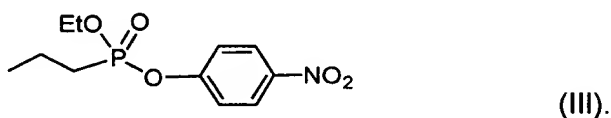
86. The method of claim 81, wherein the display moiety is a polypeptide.

5 87. The method of claim 82, wherein the display moiety is a polynucleotide.

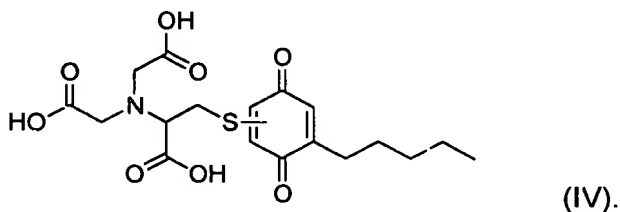
88. The method of claim 81, wherein the reactant ligand is a moiety of formula (II):



10 89. The method of claim 81, wherein the reactant ligand is a moiety of formula (III):



90. The method of claim 81, wherein the reactant ligand is a moiety of formula (IV):



15 91. The method of claim 81, wherein the surface is selected from the group consisting of sepharose, agarose, polyacrylamide, polystyrene,

dextran, lipid monolayer, lipid bilayer, metal, metal oxide, glass, ceramic, quartz, silicon, polyethylene, and polypropylene.

92. The method of claim 81, wherein the surface comprises a gel.

93. The method of claim 81, wherein the surface comprises a porous material.

94. The method of claim 81, wherein the surface comprises gold.

95. The method of claim 94, wherein the reactant ligand is attached to the surface by a moiety of formula (XIII):



wherein -L- is $-(A_x-B_y-E_z-D)_w$;

each A, B, E and D are individually $C(R_A R_A')$ -, $-C(R_B R_B')$ -, $-C(R_E R_E')$ -, and $-C(R_D R_D')$ -, respectively;

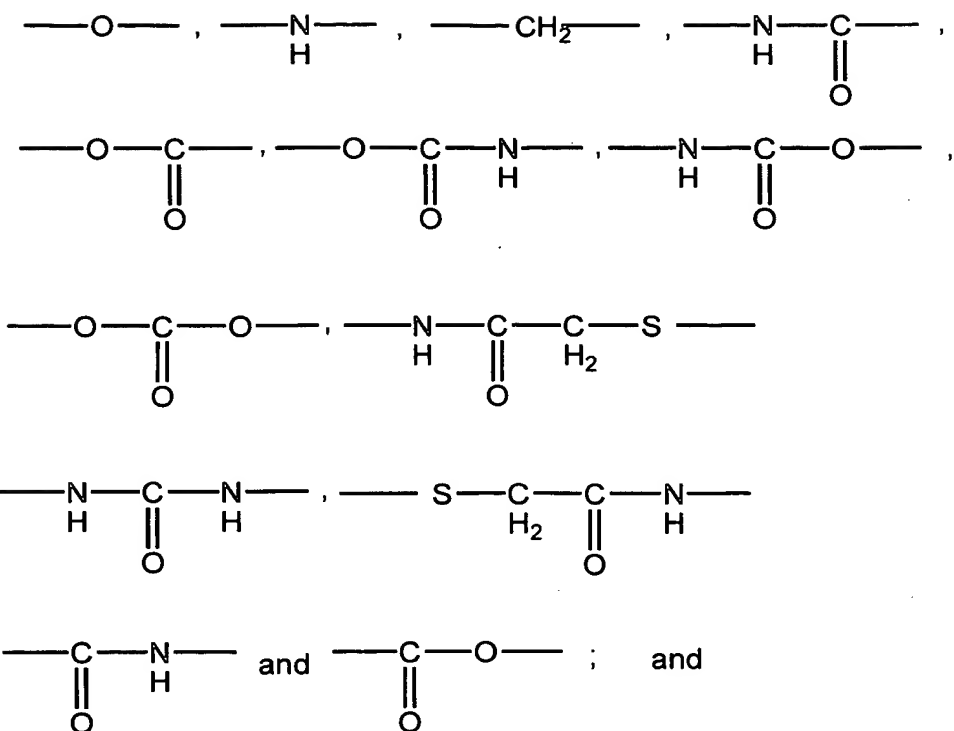
each R_A , R_B , R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A , R_B , R_E and R_D together form a bond, or any two of R_A , R_B , R_E and R_D together with the atoms to which they are bonded form a ring;

each R_A' , R_B' , R_E' and R_D' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A' , R_B' , R_E' and R_D' together form a bond, or any two R_A' , R_B' , R_E' and R_D' together with the atoms to which they are bonded form a ring;

each x, y and z are individually either 0 or 1;

w is 1 to 5;

-Q- is selected from the group consisting of



-T comprises said reactant ligand.

96. The method of claim 95, wherein -L- contains 8 to 18 carbon atoms.

5 97. The method of claim 95, wherein -L- is an alkylene containing 6 to 18 carbon atoms, and -Q- is -O-.

98. The method of claim 95, wherein -Q- is -O- or -CH₂-.

10 99. A method of attaching a polypeptide to a surface, comprising:
non-covalently attaching a polypeptide to a reactant ligand
specific to the polypeptide; followed by
forming a covalent bond between the polypeptide and the
reactant ligand.

100. The method of claim 99, wherein the half-life of the covalent bond is at least 3 minutes.

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101. The method of claim 99, wherein the half-life of the covalent bond is at least 30 minutes.

102. The method of claim 99, wherein the half-life of the covalent bond is at least 1 hour.

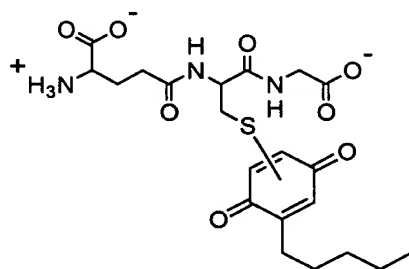
5 103. The method of claim 99, wherein the half-life of the covalent bond is at least 24 hours.

104. The method of claim 99, wherein a fusion comprises the polypeptide and a display moiety.

10 105. The method of claim 104, wherein the display moiety is a polypeptide.

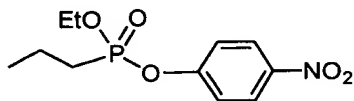
106. The method of claim 104, wherein the display moiety is a polynucleotide.

107. The method of claim 99, wherein the reactant ligand is a moiety of formula (II):



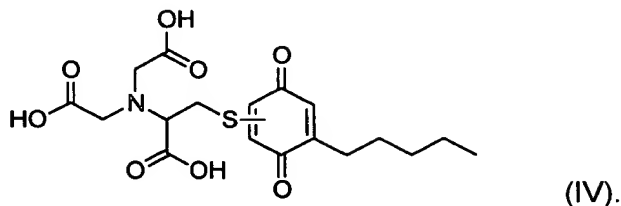
(II).

108. The method of claim 99, wherein the reactant ligand is a moiety of formula (III):



(III).

109. The method of claim 99, wherein the reactant ligand is a moiety of formula (IV):



110. The method of claim 99, wherein the surface is selected from the group consisting of sepharose, agarose, polyacrylamide, polystyrene, dextran, lipid monolayer, lipid bilayer, metal, metal oxide, glass, ceramic, quartz, silicon, polyethylene, and polypropylene.

111. The method of claim 99, wherein the surface comprises a gel.

112. The method of claim 99, wherein the surface comprises a porous material.

113. The method of claim 99, wherein the surface comprises gold.

114. The method of claim 113, wherein the reactant ligand is attached to the surface by a moiety of formula (XIII):



wherein -L- is $-(A_x-B_y-E_z-D)_w$;

each A, B, E and D are individually $C(R_A R_A')$ -, $-C(R_B R_B')$ -, $-C(R_E R_E')$ -, and $-C(R_D R_D')$ -, respectively;

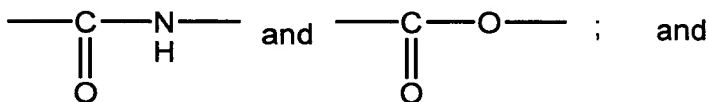
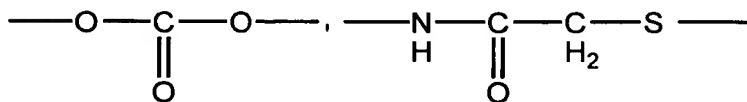
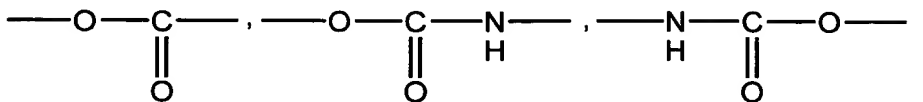
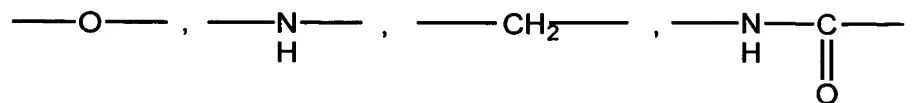
each R_A , R_B , R_E and R_D is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A , R_B , R_E and R_D together form a bond, or any two of R_A , R_B , R_E and R_D together with the atoms to which they are bonded form a ring;

each R_A' , R_B' , R_E' and R_D' is selected from the group consisting of H, alkyl, alkenyl, alkynyl, aryl and heterocyclic radical, or any two of R_A' , R_B' , R_E' and R_D' together form a bond, or any two R_A' , R_B' , R_E' and R_D' together with the atoms to which they are bonded form a ring;

each x, y and z are individually either 0 or 1;

w is 1 to 5;

-Q- is selected from the group consisting of



-T comprises said reactant ligand.

115. The method of claim 114, wherein -L- contains 8 to 18 carbon atoms.

116. The method of claim 114, wherein -L- is an alkylene containing 6 to 18 carbon atoms, and -Q- is -O-.

117. The method of claim 114, wherein -Q- is -O- or -CH₂-.

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